

# Organic nitrogen distribution in seven Tunisian soil types under contrasting pedogenetic conditions

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**Abstract** The study of nitrogen organic matter is of great importance for both economic and environmental reasons. Thus, seven soils developed under different pedogenetic and bioclimatic conditions in Tunisia were selected to investigate the distribution of organic nitrogen (ON). Our results showed an enrichment in ON of superficial horizons to the detriment of the deep horizons. Nevertheless, this decrease in ON contents with depth occurred following different patterns according to soil type. Moreover, the investigation of ON distribution in clay- and silt-sized particles (below 50 µm) indicated a considerable contribution of the fine fraction (65–98% of the soil total ON) to the ON immobilization in soils, especially in the deep horizons, while the coarse fraction (>50 µm) seems to play a less important role. The isolation of humic substances (Humic Acids, Fulvic Acids and Humin (H)) from the fine fraction and the analysis of the ON in each of these organic fractions permitted to conclude the majority of soil ON is incorporated within the H, thus suggesting a low bioavailability of nitrogen in the studied soils.

**Keywords** Soil fine fractions · Humic fractions · Organic matter · Organic carbon and nitrogen

## Introduction

In arid and semiarid Mediterranean regions, N is the most limiting nutrient in soils and one of the most expensive inputs applied in agricultural systems. It plays an important role in soil fertility and land productivity (St. Luce et al. 2011). Indeed, N is an integral part of all proteins and is one of the main chemical elements required for plant growth and photosynthesis. It is also a component of energy transfer compounds, which allows cells to conserve and use the energy released in metabolism. Besides, N is a significant constituent of nucleic acids that allows cells, and eventually whole plants, to grow and reproduce (Ohyma 2010). In agricultural lands, availability of N is considered as the most limiting factor of crop growth. In fact, more than 90% of N stored in soils is typically in organic form not available to crop and only a small percentage of ON (about 1–4%) is mineralized to simple inorganic N forms ( $\text{NH}_4^+$  and  $\text{NO}_3^-$ ) that can be absorbed by plants (Vimlesh and Giri 2011). Thus, utilization of organic N by crop requires prior transformation by soil microbes to inorganic available forms. Nevertheless, these microbial processes are not instantaneous and depend on many conditions such as the influence of pedo-climate, soil type, pedogenetic characteristics and physicochemical properties specific to the soil (Bechtold and Naiman 2006). In addition to that, soils of arid regions are generally poor in N because of the naturally weak organic restitutions, principal source of N in soil. Thus, in order to solve this deficit, supplementing cultivated crops with chemical nitrogenous fertilizers has become a necessity (Schröder 2014), especially knowing that mineralization of ON in soils is too slow to meet the entire needs of crops (Schipper et al. 2004).

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In Tunisia, for instance, N fertilization constitutes the most important agronomic practice for sustaining growth and improving maximum economic yield (Ayadi et al. 2014). In the world, the increase in agriculture production resulting from the green revolution was mainly based on the use of mineral fertilizers, which has led to a global N fertilizer consumption increase from 10.8 Mt in 1960 to 85.6 Mt in 2000 (Ayadi et al. 2012). Therefore, over the last two decades, most of the researches on the use of organic and mineral fertilizers emphasized their N fertilizing value (Barabasz et al. 2001). Recently, new concerns have been raised about the adverse effects of N on the environment mainly because of high application rates of N fertilizers and improper land management practices (St Luce et al. 2011). Groundwater contamination by nitrates has become a worldwide concern (Mulvaney et al. 2001). Thus, to improve the efficiency of N uptake by plants and reduce massive use of N mineral fertilizers, we need an accurate prediction of N supply from soil organic matter (SOM) in both natural and agricultural ecosystems (Bhagal et al. 2001). A major focus of agronomists and soil scientists today is the study of ON occurrence and distribution in soils through a multitude of approaches (NOM characterization, SOM fractionation, humic substances extraction, etc.) (Wander 2004). Consequently, innumerable researchers have studied N dynamic in soil for a long time. Nevertheless, most of these studies have examined ON in soil surface horizons only with little attention given to NOM distribution according to depth. Besides, these works were conducted mostly on soils developed under temperate climate (Schmidt and Kögel-Knabner 2002; Kölbl and Kögel-Knabner 2004; Abdul kader 2006) or in subtropical and tropical regions (Sano et al. 2004; Jiang et al. 2011; Yan et al. 2012). Only few researchers have investigated this issue in soils of arid and semiarid regions, which makes N management in such areas difficult and demands for fundamental understanding of ON distribution. For example, Caravaca et al. (1999) investigated the distribution of ON in the fine fraction of soil samples taken from top 20 cm in both cultivated and forest soils developed under semiarid bioclimate (south Spain). The authors showed that the cultivated soils had greater percentage of total soil N in the fine fraction than the soils under natural vegetation. The authors reported that this distribution could lead to a decline in soil productivity since the finest and most fertile soil particles are those which are generally removed by water erosion and unsuitable agricultural management in semiarid regions.

Against this backdrop, the present research was undertaken with the aim of better identifying and understanding ON distribution in the different horizons, particle size and organic fractions of seven different soil types of Tunisia.

## Materials and methods

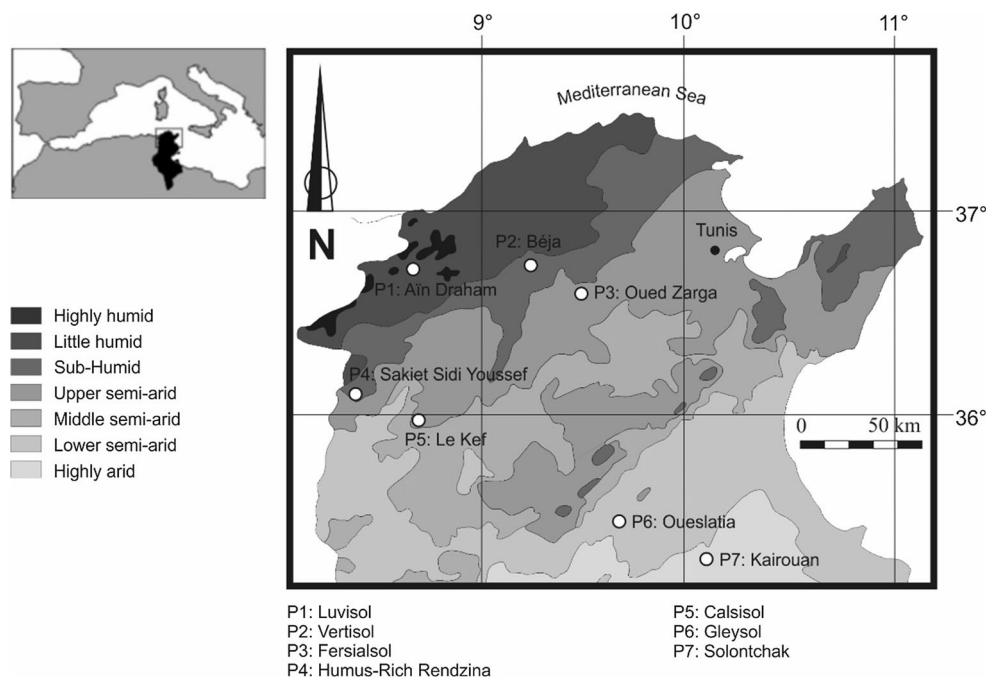
### Soil sampling and characterization

We selected for this study seven soils developed under contrasted bioclimatic and geological conditions in northern and central Tunisia (Fig. 1): We sampled a Luvisol under forest vegetation (*Quercus ilex* and *Quercus suber*) on a naturally well-exposed soil located in the Ain Draham rainy area. This region has a humid bioclimate with tempered winter. The average monthly air temperature is 15 °C ranging from 6.6 (January) to 23.9 °C (July), and the average annual rainfall is around 1534 mm. We have taken a Vertisol and a Fersialsol, respectively, from the Béja and Oued Zarga agricultural area. They were both cultivated (cereal crops). It is a very narrow zone, but with a significant pedological and bioclimatic variability ranging from a sub-humid Mediterranean bioclimate with wet winter and dry summer (Béja) to a middle semiarid bioclimate (Oued Zarga). The average monthly air temperature of the region is about 18 °C ranging from 9.3–9.5 °C (January) to 26.9–27.3 °C (July), and the average annual rainfall is about 550 mm. We collected a humus-rich Rendzina and a Calcisol developed under forest species (*Pinus halepensis* and *Quercus ilex*), respectively, in the Sakiet Sidi Youssef and Kef area constituting the uppermost part of north-western Tunisia. The bioclimate of this zone is the sub-humid bioclimate with mild winter. The monthly air temperature of Sakiet Sidi Youssef is 14 °C ranging from 5.7 (January) to 24.3 °C (July), and the average annual rainfall is around 770 mm. We sampled also a Gleysol in the Oueslatia region characterized by a natural vegetation dominated by *Pinus halepensis*, *Pistacia lentiscus* and *Olea europaea*. The region has a lower semiarid bioclimate with an average monthly air temperature about 16.7 °C ranging from 7.8 (January) to 26.6 °C (July), and an average annual rainfall around 400 mm. Finally, we collected a Solontchak covered by a halomorphic vegetation from Kairouan region. The bioclimate of this zone is the highly arid with an average monthly air temperature of 19.5 °C ranging from 10.4 (January) to 28 °C (July), and the average annual rainfall is around 300 mm.

For all sites, we have taken soil samples from each horizon of the profile. Then, soil samples were air dried, sieved through a 2-mm-diameter mesh and stored in sealed containers at 4 °C.

We characterized the soil samples for particle size distribution, total organic carbon (TOC), total organic nitrogen (TON), lime (CaCO<sub>3</sub>) contents, electrical conductivity (EC) and pH according to French and international norms (AFNOR 1995a, b, c, 1998, 2003, 2005). Moreover, we submitted the studied soils to a particle size fractionation to

**Fig. 1** Localization of the study sites according to bioclimatic stages



isolate the soil fine fraction below 50  $\mu\text{m}$ . The humic substances of this fraction were equally isolated. All separates (soil fine fractions and humic substances) were quantified for their TON and TOC contents.

### Particle size fractionation

Fifty grams of soil sample was dispersed in 250 mL of distilled water and submitted to 5-min ultrasonic treatment in continuous mode to ensure soil particles homogenization and dispersion. The used sonicator is a heat system ultrasonics model W225R (20 kHz frequency, 100 W power). The suspension was then filtered through a 50- $\mu\text{m}$  filter under a distilled water jet and the fractions below and above 50  $\mu\text{m}$  recovered in distilled water. Each fraction was later dried in an oven under 50  $^{\circ}\text{C}$  in order to reach constant weight. The different fractions are finely crushed to be analyzed for TOC and TON.

### Humic substances extraction

Humic substances were extracted from the soil fraction smaller 50  $\mu\text{m}$  only. Ten grams of soil (<50  $\mu\text{m}$ ) was suspended in 50 mL of  $\text{Na}_4\text{P}_2\text{O}_7$  solution (0.1 M, pH 9.8) and shaken for 1 h. Then, the suspension was centrifuged (12,000 tr/min for 20 min), and the supernatant was collected. The remaining residue is mixed with 50 mL NaOH (0.1 M, pH 12) and centrifuged after 1 h of shaking, and a second supernatant is collected. The obtained insoluble residue is the H. Then, both supernatants extracted by  $\text{Na}_4\text{P}_2\text{O}_7$  and NaOH are mixed in equal volumes, acidified

using HCl (2 N, pH 1.5) and the suspension allowed to stand for 12 h before being centrifuged. The supernatant contains FA, while the precipitate forms the HA which are redissolved in a 0.01 N NaOH solution. This procedure is largely documented, and more details can be found in the literature (Olk et al. 1998). The different extracted humic fractions (HA, FA and H) were analyzed for TON and TOC.

## Results and discussion

### Correlations between soil properties and TON content

The main physicochemical properties of the studied soils are presented in Table 1. The examination of these data shows an important variability among soils and horizons. For instance, large differences were recorded concerning soil richness in organic matter (OM), as OC contents ranged from 0.16% in the Gleysol to 11% in the Rendzina. Nevertheless, different OM distribution patterns were identified. Indeed, the Solontchak registered low OM contents, which do not exceed 0.5% equally distributed throughout the profile, while OM values decreased rapidly with the depth in the case of the Rendzina. OC contents in this soil fell from 11% top to 1.8% bottom. Moreover, the various studied soils showed comparable discontinuities between surface and deep horizons. This appears clearly in the vertical profile of OM characterized by a decrease in TOC and TON contents as soil depth increases. Thus, for

**Table 1** Selected physicochemical properties of the studied soils

Soil	Depth (cm)	pH	EC (mS.cm <sup>-1</sup> )	Sand (%)	Silt (%)	Clay (%)	CaCO <sub>3</sub> (%)	TON % <sup>a</sup>	TOC % <sup>b</sup>	C/N	C/N <sub>HA+FA</sub> <sup>c,d</sup>	C/N <sub>H</sub> <sup>e</sup>
Luvisol	0–20	6.1	0.2	62.00	17.00	21.00	1.0	1.68	2.43	14.4	8.3	6.9
	20–60	6.7	0.1	63.00	21.01	18.00	6.5	0.95	1.00	10.5	10.2	13.3
	60–130	4.8	0.1	19.00	10.00	68.00	1.0	1.14	0.69	6.0	14.1	1.6
	130–200	5.1	0.1	63.00	2.00	33.00	3.2	0.68	0.41	6.0	17.2	4.3
Vertisol	0–30	8.3	0.3	26.00	42.00	29.00	30.5	2.14	2.58	12.0	5.8	8.6
	30–54	8.2	0.3	29.00	49.00	23.00	28.2	1.84	1.52	8.2	4.1	12.7
	54–122	8.1	0.9	28.00	53.00	20.00	77.6	0.36	0.24	6.6	15.0	15.2
Ferralsol	0–26	7.9	0.2	47.00	37.00	17.00	2.3	1.49	1.43	9.6	5.7	10.7
	26–102	7.8	0.1	18.00	21.00	60.00	0.0	1.18	0.50	4.2	17.3	1.0
	102–180	8.2	0.2	42.00	34.00	24.00	36.4	0.54	0.17	3.1	1.2	4.5
	180–200	7.9	1.3	41.00	33.00	24.00	51.7	0.39	0.19	4.8	0.4	20.0
Humus-rich Rendzina	0–15	8.3	0.3	26.45	38.05	34.80	27.0	7.00	11.10	15.9	14.9	14.3
	15–25	8.2	0.2	24.64	30.91	39.90	42.3	3.10	3.56	11.5	15.2	9.7
	25–55	8.1	0.3	25.01	24.97	36.22	57.9	1.50	2.12	14.1	15.4	8.8
	55–73	8.1	0.3	24.50	26.50	45.00	61.3	1.40	1.80	12.8	16.6	7.2
Calcisol	0–20	7.9	0.3	19.17	45.37	36.90	7.0	3.40	3.71	10.9	14.1	10.6
	20–45	8.2	0.2	20.00	36.59	44.23	8.1	2.80	3.30	11.7	11.8	10.2
	45–115	8.4	0.3	25.32	32.80	39.40	22.0	2.40	2.50	10.4	9.5	12.8
Gleysol	0–3	7.9	0.4	37.00	43.00	21.00	50.0	1.78	1.82	10.2	10.6	9.3
	3–18	8.0	0.4	40.00	45.00	14.00	55.5	1.70	2.37	13.9	20.0	10.8
	18–48	8.0	0.3	42.00	38.00	22.00	51.1	1.65	1.57	9.5	20.0	6.7
	48–78	8.0	0.5	33.00	35.00	31.00	58.8	0.72	0.43	5.9	4.0	6.1
	78–128	8.0	0.4	25.00	44.00	31.00	66.6	0.41	0.19	4.6	5.2	4.2
	128–148	8.0	0.4	23.00	43.00	33.00	78.5	0.15	0.16	10.6	11.2	10.0
Solontchak	148–168	8.0	0.4	28.00	42.00	30.00	90.7	0.11	0.17	15.4	20.0	12.8
	0–30	7.7	4.3	31.00	34.00	35.00	33.3	0.49	0.34	6.9	9.1	4.6
	30–43	7.7	5.0	66.00	23.00	9.00	35.5	0.33	0.27	8.2	9.1	4.3
	43–113	7.8	6.4	14.00	42.00	41.00	30.0	0.70	0.49	7.0	6.0	7.3
	113–126	7.7	6.4	65.00	22.00	9.00	40.5	0.51	0.27	5.3	4.4	5.2
	126–182	7.7	8.4	19.00	44.00	37.00	58.0	0.32	0.20	6.2	7.7	5.7

<sup>a</sup> Total organic nitrogen<sup>b</sup> Total organic carbon<sup>c</sup> Humic acids<sup>d</sup> Fulvic acids<sup>e</sup> Humin

all studied soil profiles, topsoil horizons showed the highest TOC and TON values, while the deep horizons presented lower organic proportions.

As the aim of this paper is investigating factors affecting ON contents and distribution in soils, a statistical analysis was performed to find out eventual correlations between ON contents and properties of the studied soils (all horizons (30) of the seven studied soils were considered for this analysis). The results revealed a strong positive correlation between ON and OC in soils ( $r^2 = 0.950$ ,  $p < 0.0001$ ). No

other correlations were found. Nevertheless, the same statistical analysis carried out with 28 additional soil samples studied by *Dridi (2007, data unpublished)* permitted to confirm the correlation already found with OC ( $r^2 = 0.937$ ,  $p < 0.0001$ ) but showed also ON was significantly correlated with the soil clay + silt fraction ( $r^2 = 0.320$ ,  $p < 0.05$ ). This finding guided our choice in this paper to monitor ON distribution in soils according to different particle size fractions (notably the fine fraction) and among the various organic fractions (FA, HA and H).

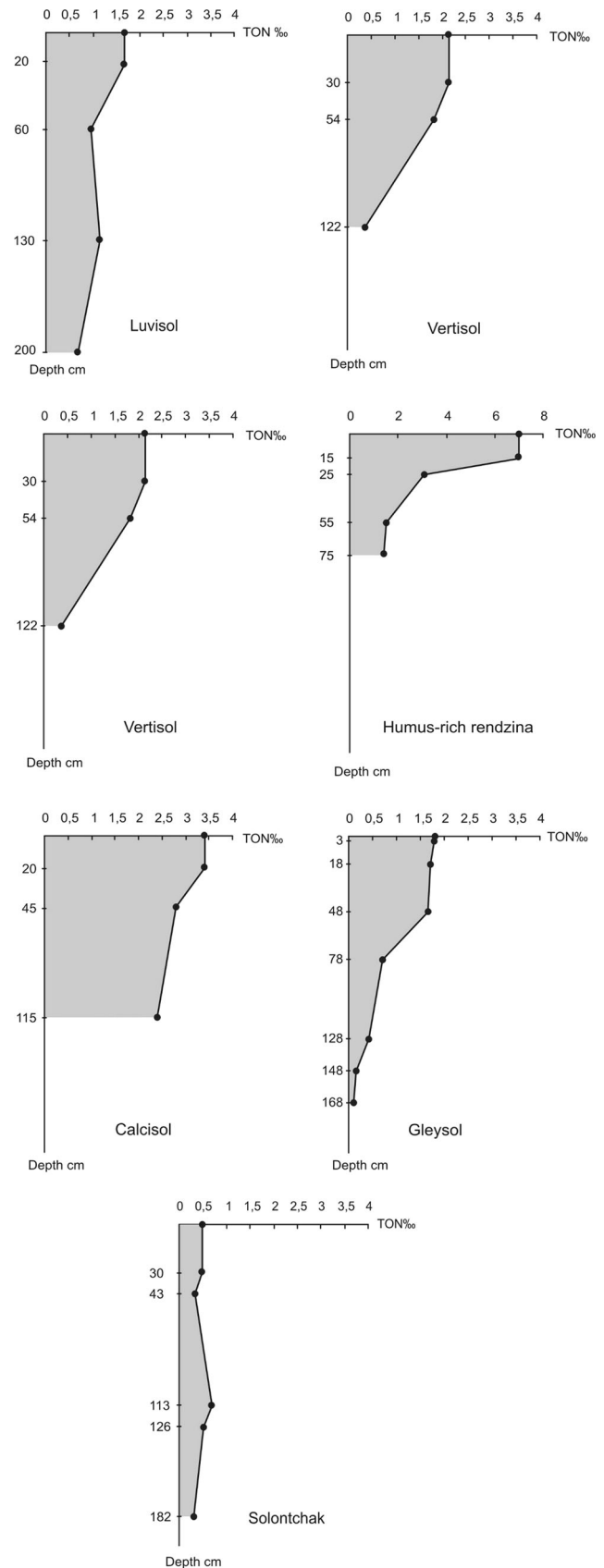
## Depth distribution of TON in soils

The vertical distributions of TON in the studied soils are reported in Fig. 2. The examination of these data shows a large variability in ON contents and vertical profiles depending on soil type. Indeed, TON values ranged from 0.4 to 7‰: The Rendzina and the Calcisol exhibited the highest TON values (7 and 3.4‰, respectively), while the lowest proportions were recorded for the Solontchak (0.4‰). This is in harmony with TOC values as the two first soils (the Rendzina and the Calcisol) are the richest in OM, while the Solontchak is the poorest. For this latter, several studies report, in accordance with what we found, salt-affected soils are generally characterized by low OM and N contents (Lodhi et al. 2009).

In addition to that, a common trend was observed for all soils, that is an enrichment in TON of superficial horizons to the detriment of the deep horizons characterized by lower ON contents. Nevertheless, this decrease in TON with soil depth occurred differently among the studied soils: For the Rendzina, the decline in TON from the surface to the deep horizons is brutal and quick as TON drops from 7 to 3.1‰ within 25 cm of depth. Indeed, Rendzina soils are typically characterized by the presence of a well-differentiated humic surface horizon rich in OM. On the opposite, for the Solontchak, ON was feeble and almost evenly distributed throughout the profile. Salts induce the deterioration of soil structure and the dispersion of colloidal particles (clays and OM) which are consequently leached throughout the soil, hence the ON pattern observed for the Solontchak (Walpole and Arunakumara 2010). For the other five soils, the transition in TON contents from the top to the bottom of the profile was more or less progressive. For the Gleysol, TON contents are neatly reduced after the 48- to 78-cm horizon, which was attributed to the presence of a calcareous accumulation that would prevent dissolved OM from moving to the underlying horizons. In the Vertisol, the vertic movements related to an opening of the swelling clay slits during the dry season and their withdrawal during the wet season contribute to the mixing of the profile top to bottom, thus helping to impregnate the whole profile with OM and notably with ON. For the Luvisol, Calcisol and Fersialsol, no particular profile was noticed but a profound incorporation of ON in the soil.

## Depth distribution of soil fine fraction ON

Our results show the range of ON contents associated with soil fine fractions ( $ON_{<50\mu m}$ ) is very broad as the soil samples were collected within a wide variety of horizons, textures and OM contents (data not shown). The highest  $ON_{<50\mu m}$  rate was observed for the Luvisol (3‰), while the lowest one was found in the Fersialsol (0.43‰). The



**Fig. 2** TON distribution in the studied soils



results also show that the richer the whole soil in TON, the higher the ON concentration of its fine fraction. Thus, the surface horizons which exhibited the highest TON contents are equally characterized by the richest fine fractions in ON. In order to assess the contribution of the fine fraction ( $<50\ \mu\text{m}$ ) to the TON capital of the soil, the ratio of the ON in the fine fraction to the TON of the whole soil was calculated.

The results are reported in Fig. 3. The examination of these data shows the fine fraction accounted for 65–98% of the soil total ON content in all the studied horizons except for the surface horizon of the Rendzina. Indeed, it is well-established SOM is preferably associated with the smallest or fine soil fractions, owing to their chemical and mineralogical characteristics favouring such union (Jocteur Monrosier et al. 1991; Schmidt et al. 2000; Makarov et al. 2004). Most of SOM may be observed in silt- and clay-sized primary organo-mineral separates where it probably occurred as a complex with the mineral constituents, thus making them the most fertile soil fractions. According to Christensen (1996), clays generally account for over 50% of SOM, while silt and clay together ( $<50\ \mu\text{m}$ ) may account for over 90%. Besides, many researches underline OM bound to the fine silt- and clay-sized fractions is more humified than that associated with the coarse fraction (Benoit et al. 2000; Schmidt and Kögel 2002).

In the case of the Rendzina, where the fine fraction contribution was only about 35%, this can be explained by the fact that for this soil, the fresh OM is mainly originated from the leaves of coniferous trees, known for their molecular recalcitrance to the biological breakdown. In addition to that, the low temperature in the Sakiet Sidi Youssef area ( $14\ ^\circ\text{C}$ ) contributes to prevent OM degradation. Thus, the humified OM fraction believed to be localized in the fine soil fraction ( $<50\ \mu\text{m}$ ) is quantitatively limited, while a majority of the fresh or less humified OM is associated with the coarse fraction. This hypothesis is corroborated by the highest C/N ratio (15.9) determined for the surface horizon of the Rendzina, which indicates OM is little humified. Moreover, an increase in the  $\text{ON}_{<50\mu\text{m}}/\text{TON}$  ratio as the soil depth increases is noticed for all the studied soils except the Vertisol, where the vertic movements would be again responsible for the homogenization of the profile. Thus, a considerable contribution of the fine fraction ( $<50\ \mu\text{m}$ ) to the ON immobilization in soils, especially in the deep horizons, was evidenced.

This increase in the concentration of ON in the fine fraction with soil depth may be explained either by an enrichment of the deep horizons in silt and clays or by a higher affinity of ON to the fine particles related to soil OM humification status. The first hypothesis was rejected as we observed no significant difference in the abundance of the fine fraction between the top and the bottom of the profile

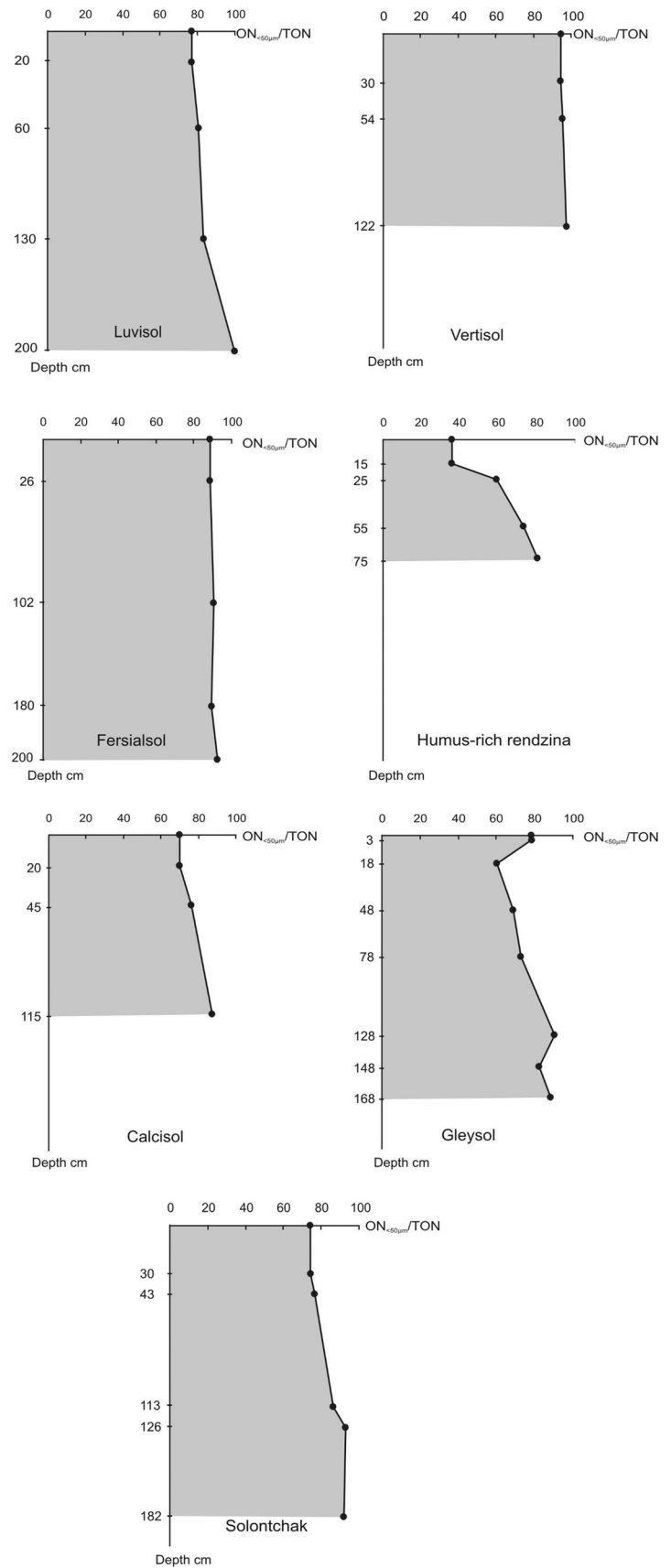
for the different studied soils (data not shown). As far as the second hypothesis is concerned, the examination of the C/N values revealed consistently lower C/N ratios with increasing soil depth, which supports the postulation OM in the deep horizons is more humified than in the soil surface. Knowing the more OM is humified, the more it is likely to be bound to silt and clays, this would be a plausible explanation of the increase in the fine fraction contribution to the total ON content of the soil with depth. The example of the Gleysol is a good illustration of this hypothesis. Indeed, the upper horizon (0–3 cm) with  $\text{C/N} \approx 10$  presented an  $\text{ON}_{<50\mu\text{m}}/\text{TON}$  rate equal to 80%. This value dropped to 60% in the 3- to 18-cm horizon whose C/N abruptly moved to  $\approx 14$ . Then,  $\text{ON}_{<50\mu\text{m}}/\text{TON}$  ratios of the underlying horizons started to increase in parallel with a decrease in their C/N. The highest  $\text{ON}_{<50\mu\text{m}}/\text{TON}$  value (90%) recorded in the 78- to 128-cm horizon corresponded to the lowest C/N (4.6). At the bottom of the profile (128–168 cm),  $\text{ON}_{<50\mu\text{m}}/\text{TON}$  ratios decreased slightly, while the C/N increased to 15, probably because of the presence, at this depth, of hydromorphic conditions which inhibited OM decomposition.

As far as N bioavailability to soil microflora and plants is concerned, the presence of large proportions of ON associated with the soil fine fraction suggests much of this N exists in a stable form, “protected” by silt and clay particles, which makes it not easily accessible for microbial degradation (Krull et al. 2003; Uzoho and Igbojionu 2014). Plants cultivated in fine-textured soils would equally have less available mineral N for their nutrition, especially if these crops present a relatively profound root system localized in the deep soil horizons where we found the highest rates of ON incorporation to the fine fraction.

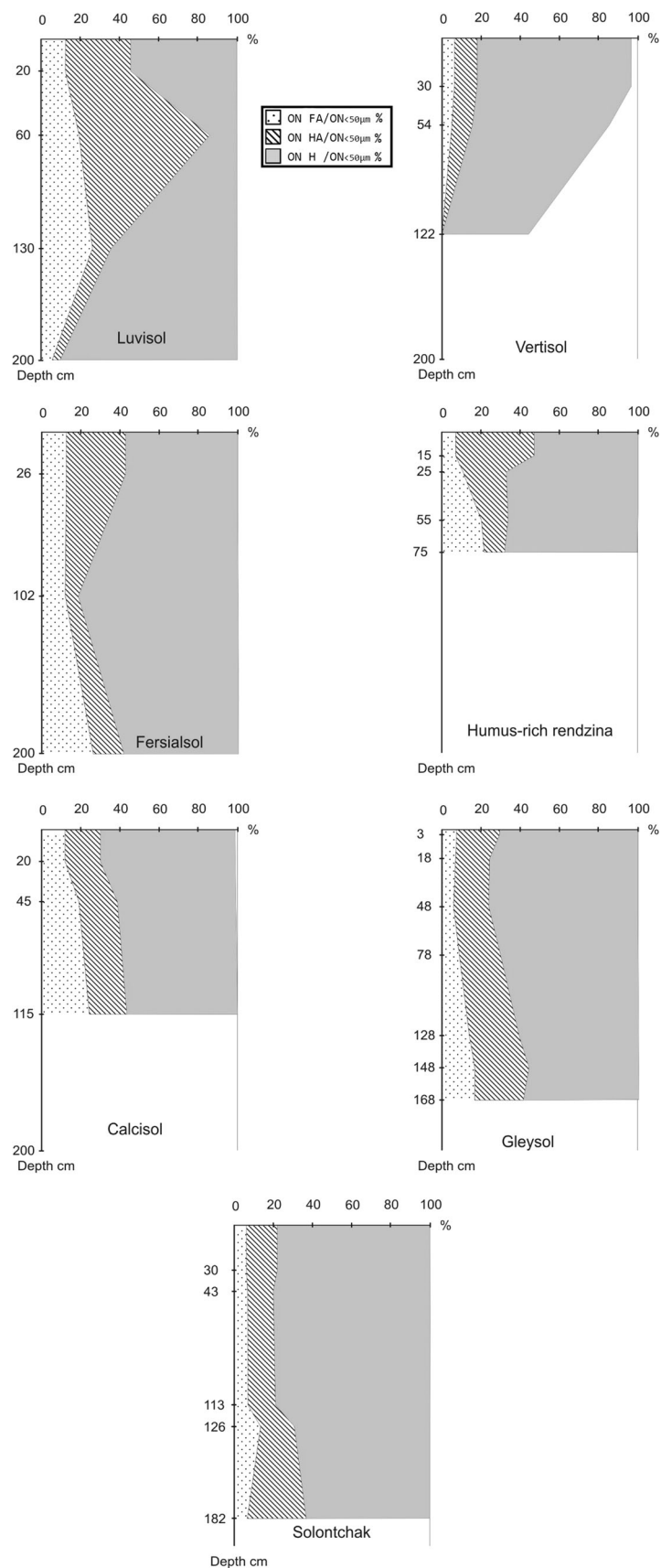
### Depth distribution of humic fractions ON

As the soil fine fraction largely contributed to the total ON content in almost all the studied horizons (65–98% of TON), and since it is well known this fraction is the richest in humified OM, the choice was made to further investigate the distribution of ON in the different humic substances extracted from this soil fraction ( $<50\ \mu\text{m}$ ). The contribution of each humic fraction (HA, FA and H), expressed as percentage of the ON content of the fine fraction, is reported in Fig. 4. The analysis of these data clearly indicates that most of ON in all the studied soils is associated with the humin fraction. This enrichment of the humin in ON is probably the result of a preferential accumulation of microbial proteinaceous materials (proteins, polypeptides, peptides and amino acids) due to entrapment in the voids of the three-dimensional structure of humin and to physical and chemical retention of N compounds by the humin surface (Schulten and Schnitzer 1997). Besides, ON

**Fig. 3** ON distribution in soil fine fraction ( $<50\ \mu\text{m}$ )



**Fig. 4** ON distribution in humic fractions (*FA* fulvic acids, *HA* humic acids, *H* humin)





incorporated in humin is likely more stable because humin presents high thermal stability and resistance to acid hydrolysis. Indeed,  $ON_H/ON_{<50\mu m}$  ratios ranged from 52 to 90% for all the studied horizons except for the lower horizons of the Vertisol (44%) and the Fersialsol (22%) and the 20- to 60-cm horizon of the Luvisol (14%). For these three horizons, the relatively low ON content of the humin can be attributed to the maturation degree of this humic fraction. In fact,  $C/N_H$  calculated for the above-cited horizons is the highest in comparison with the other horizons of each soil ( $C/N_H = 13$  for the Luvisol,  $C/N_H = 20$  for the Fersialsol,  $C/N_H = 15$  for the Vertisol, cf. Table 1). These high ratios indicate a feeble incorporation of the ON probably related to a low degree of condensation of the humin. In the Luvisol, the 20- to 60-cm layer corresponds to the eluvial horizon continuously leached and then impoverished in nutrients and OM. Thus, the humin in this horizon is exhibiting a less important degree of maturation because of the lack of OM. Regarding the Fersialsol, the lower horizon (180–200 cm) is an encrusted horizon presenting a  $CaCO_3$  accumulation (51%). The presence of this solid crust led us to suppose the humin contained in this horizon is rather inherited. The 54- to 122-cm horizon of the Vertisol equally exhibits a high  $CaCO_3$  content (77%). For this horizon, the evolution of the humin is likely depressed by  $CaCO_3$  (1) because of  $Ca^{2+}$  cations which saturate the free sites of OM, thus inhibiting its polymerization or (2) through crystalline coating of OM by calcium carbonates (Bouagila and Gallali 2008).

Regarding N availability, our findings indicate ON highly incorporated in the humin is under a very stable form, highly recalcitrant to biodegradation. Thus, important amounts of the ON in the studied soils could difficultly be mineralized to become accessible to plants. This is an important matter to be considered by farmers and managers when planning N fertilization, as they can rely on very little supplies of mineral N originating from the mineralization of SOM.

## Conclusion

Nitrogen plays a paramount role in soil fertility and agricultural land productivity, hence the importance of understanding ON distribution, especially in the semiarid and arid soils known for their low OM and N contents, which makes N management in such areas difficult and demands for better fundamental comprehension of ON dynamics. Therefore, a combination of numerous physicochemical analyses, particle size fractionation and humic substances fractionation was used to investigate ON contents and distribution in seven different soils of Tunisia, developed under contrasting bioclimatic and pedogenetic conditions.

Our results showed a large variability in ON contents and vertical profiles depending on soil type. An enrichment in ON of the superficial horizons to the detriment of the deep horizons was observed among the studied soils. Nevertheless, this enrichment occurred following different patterns according to soil nature (abruptly or progressively). Moreover, the investigation of ON distribution in clay- and silt-sized particles (below 50  $\mu m$ ) indicated a considerable contribution of the fine fraction (65–98% of the soil TON) to the ON immobilization in soils, especially in the deep horizons, while the coarse fraction ( $>50 \mu m$ ) seems to play a less important role. The isolation of humic acids, fulvic acids and humin from the fine fraction OM and the analysis of the ON of each of these organic fractions permitted to conclude the majority of soil ON is incorporated within the humin, highly recalcitrant to biodegradation. Consequently, as far as N bioavailability is concerned, the presence of large proportions of ON associated with the soil fine fraction and mainly incorporated within the humin suggests much of this N exists in a very stable form, which makes it not easily accessible for microbial degradation. Thus, a low bioavailability of N to microflora and crops is expected for the studied soils. In order to check this assumption, accurate prediction of N mineralization dynamics in soil is needed. Indeed, when developing fertilization strategies, the calculation of the appropriate amounts of organic or chemical N fertilizers to be added to soil must take into account the quantities of mineral nitrogen supplied by the mineralization of soil ON stocks, especially that in arid and semiarid countries, farmers tend to apply excessive doses of nitrogenous fertilizer in an unbalanced way. The described differences in ON contents and dynamics shown in this work suggest different ON mineralization patterns may equally exist and emphasize more the need of an accurate assessment of ON mineralization in the studied soils. A research conducted in the same soils presented in this study and coupling field and laboratory mineral nitrogen monitoring is currently ongoing. The findings of this study would allow to elaborate concrete recommendations concerning soil N fertilization.

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